SIAM WEED BASED HYDROGEL HYBRID FOR TISSUE ENGINEERING

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To my beloved parents, husband, family and friend

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In the name of Allah the Most Compassionate, the Most Merciful. Salawat and Salaam to our beloved prophet Muhammad SAW. With the completion of this thesis report praise to Allah, by Him who given the chances and wisdom.

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ABSTRACT

Tissue engineered skin grafts have been considered as an auxiliary for replacement of damaged skin due to burns and injury. In previous studies, gelatin hybrid with natural remedy were used in order to create 3D environment for the skin grafts. However, these gelatin hybrids were not sufficient to promote the healing process especially for infected wounds due to lack of therapeutic properties and their mechanical properties are yet to be defined. In this research, Siam Weed therapeutic properties promote healing process and its mechanical properties were enhanced by gelatin and electrospinning technique to produce Siam weed-gelatin electrospun scaffold as a successful 3D skin tissue engineering grafts. The effects of content of Siam weed extracts on the electrospun scaffolds are determined using scanning electron microscopy, Fourier Transfom Infrared spectroscopy and degradation study of solution properties. The results indicated that concentration affects the solution properties such as viscosity, conductivity and ultimately the fiber diameter. The fibrous meshes were then crosslinked with glutaraldehyde vapor and its mechanical properties were determined by tensile . The value of tensile strength of the crosslinked fibrous scaffold was higher than that of human skin proving that it has high potential to be a successful skin graft when immobilized at the wound site. Cell proliferation study of the Siam weed extracts indicated that the extracts had significantly stimulate cell proliferation with a strong response at the concentration of 25 µg/ml therefore giving the optimum proliferation of the cells. The results implied the possibility of using Siam weed-gelatin fibrous scaffold as a promising candidate for tissue engineered skin grafts

ABSTRAK

Kejuruteraan cantuman tisu kulit dijadikan sebagai satu pilihan untuk menggantikan kulit yang rosak diakibatkan oleh kebakaran atau kecederaan. Menurut kajian terdahulu, gelatin yang digabungkan dengan bahan semulajadi telah digunakan untuk menghasilkan persekitaran 3 dimensi untuk cantuman kulit. Bagaimanapun, gelatin yang digabungkan dengan bahan semulajadi tidak mampu untuk meningkatkan proses penyembuhan terutamanya kepada jangkitan luka disebabkan oleh kurangnya sifat terapeutik malah sifat mekanikal juga masih lagi tidak diketahui. Dalam penyelidikan ini,penggunaan *Siam weed* mampu meningkatkan sifat penyembuhan dan sifat mekanikalnya turut ditingkatkan dengan penggabungan bersama gelatin dan proses *electrospinning.* Teknik ini menghasilkan perancah Siam weed-gelatin sebagai satu tisu kulit yang mempunyai persekitaran 3 dimensi yang berjaya. Kesan *Siam weed* ke atas serat ditentukan menggunakan *scanning electron microscopy, Fourier Transform Infrared spectroscopy* dan dehgradasi sifat larutan. Hasil keputusan mendapati kepekatan larutan mempengaruhi sifat larutan seperti kelikatan, kekonduksian dan diameter serat. Serat yang dihasilkan di gabungkan dengan wap *glutaraldehyde* dan sifat mekanikalnya di tentukan oleh ujian mekanikal. Nilai kekuatan terikan serat yang telah digabungkan dengan wap *glutaraldehyde* adalah lebih tinggi berbanding kulit manusia. Ini membuktikan bahawa ia mempunyai potensi yang tinggi untuk berjaya sebagai cantuman kulit.Kajian terhadap percambahan sel menggunakan ekstrak *Siam weed* menunjukkan bahawa ekstrak *siam weed* telah berjaya merangsang percambahan sel pada kepekatan 25 µg/ml. Hasil kajian ini telah berjaya membuktikan kemampuan dan potensi perancah *Siam weed*-gelatin sebagai cantuman kulit.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Tissue engineered skin grafts have been considered as an auxiliary for replacement of damaged skin due to burns and injury. There are a few current treatments for injury such as the use of medical needling technique for improvement of scar quality, non-cultured autologous skin cell suspension for repigmentation after burn [1], small fiber neuropathy induced by resiniferatoxin to promote sensory neuropathy after third degree burn [2] and the use of a novel chlorhexidine acetate nanoemulsion (CNE) against skin burn wound methicillin-resistant *Staphylococcus aureus* (MRSA) infections [3]. These treatment have been proven to improve the repigmentation of burn scars, promote both mechanical and thermal hypoalgesia of the skin and CNE as potential antimicrobial candidate for skin burn wound MRSA infections respectively [1-3]. However, these treatments have their limitations such as increasing the risk of failure of cell proliferation, infection, pain and wound healing problems with new scaring besides its expensive procedure [1] and even take too much time to heal after treatment for example 14 days healing process after the treatment of small fiber neuropathy induced by

resiniferatoxin [2]. Therefore, it is crucial to overcome these limitation by providing a practice that can meet the criteria of a successful tissue engineered practice such as having a faster wound healing process, cheaper and can promote mechanical properties by developing three dimensional (3D) grafts in order to reduce the risk of failure in cell proliferation.

A standard practice of tissue engineering is seeding cells on a scaffold. Microenvironment provided by the scaffolds will conduct the regeneration and proliferation of the cells. Factors of regeneration include the selection of materials, microstructure of the scaffolds and also bioreactor that involves mechanical loading [4].

In previous study, the natural materials used in 3D cell culture includes collagen and starch [6], gelatin-chondroitin sulfate scaffold [7], cross-linked cellulose-hyaluronic acid [8] and collagen-glycosaminoglycan [9] to promote cell proliferation and regeneration. However, these natural materials were not sufficient to promote healing process as many of these materials do not present therapeutic activities such as antibacterial and antiseptic characteristics [10] while Siam Weed extract has a very convincing therapeutic activities that includes antibacterial, anti-inflammatory, antioxidant and wound healing activity [11-16].

Many currently studies in tissue regeneration have relied on two dimensional (2D) cell culture models that fail to replicate the *in vivo* cellular [17]. Conventional cell culture provides 2D space that limits the cell growth, regeneration and proliferation [18]. In contrast, human tissues grow in 3D environment surrounded by extra-cellular matrix and cells as compared in Figure 1.1. The requirement of 3D environment motivates the use of synthetic or natural hydrogel that are able to create 3D environment in cell culturing platforms. 3D environment can be achieved by nanofibers produced by electrospinning technique. Further, the nanometer fibers can also enhance its mechanical properties for a successful cell proliferation and regeneration [19].

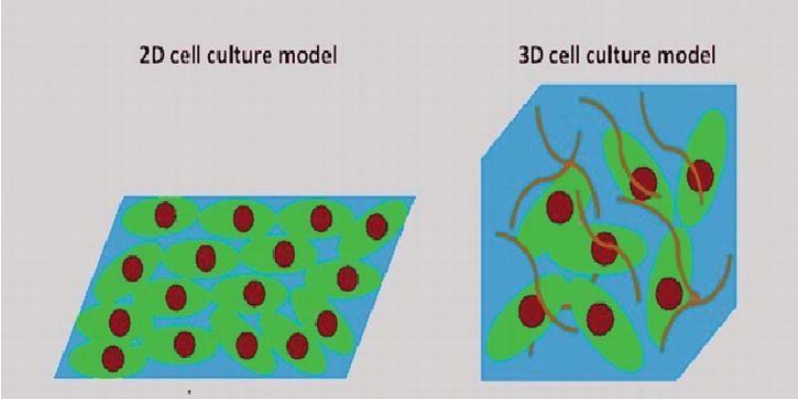

Figure 1.1: Simplified sketch of 2D and 3D cell culture (Images taken from [20])

This study aims to develop Siam weed extract-gelatin crosslinked electropsun scaffold that has therapeutic properties from Siam weed and adequate mechanical properties such as possessing excellent fiber diameter using electrospinning technique in order to develop a successful 3D tissue engineered skin grafts.

1.2 Problem Statement

Challenges in tissue engineering graft are the difficulty in preparing the scaffolds to have therapeutic properties to promote healing process and adequate mechanical properties [5] by developing three dimensional (3D) grafts imitating natural tissue.Gelatin hybrid with natural remedy was used in order to create 3D environment for cell seeding [6-9]. However, these gelatin hybrids were not sufficient to promote the healing process especially for infected wounds due to lack of therapeutic properties [10]. Besides, gelatin has poor mechanical properties that limit their implementation as dressing products [10]. In this research, Siam Weed has been added in gelatin to provide therapeutic properties for promoting proliferation process. The Siam Weed based hydrogel was spun into nanofibers to enhance its mechanical properties using an electrospinning technique. The newly developed Siam weed based gelatin nanofibers are potential to be a successful 3D skin tissue engineering grafts.

1.3 Objective of Study

The objectives for this research are as follow:

i. To determine the effect of Siam weed aqueous extract concentrations on morphology, diameter of the electrospun fibres, viscosity and conductivity, degradation behavior of Siam weed aqueous extract-gelatin solution.
ii. To investigate the effect of Glutaraldehyde crosslinking on morphology, pore size, electrospun fibre diameter, weight loss degradation profile and tensile properties of Siam weed aqueous extract-gelatin crosslinked electrospun scaffold
iii. To evaluate the cell proliferation behavior of 25, 50, 100 and 200 µg/ml concentration Siam weed aqueous extract.

1.4 Scope of Study

The scope of this research includes:

i. In the development of Siam weed based electrospun scaffolds, several types of gelatin solutions with different concentration were used to spin nanofibers in order to get microstructure without existence of beads.
ii. Two types of extracts studied in this project include Siam weed aqueous extract and ethanol extract.
iii. Four concentrations of Siam weed aqueous extracts used to spin the nanofibers include 20 µg/ml, 25 µg/ml, 30 µg/ml and 35 µg/ml. Each concentration of Siam weed aqueous extracts were mixed with gelatin to produce Siam weed based gelatin solutions.
iv. Siam weed gelatin solutions were electrospun into nanofibrous scaffolds using an electrospinning technique and cross-linked with saturated glutaraldehyde (GTA).
v. Material characteristic involves in this study include imaging of microstructure morphology and mechanical testing.

vi. The morphology of the electrospun scaffolds were characterized by using Scanning Electron Microscope (SEM) and analyzed by imageJ software.

vii. The mechanical characterization involves measuring tensile properties of cross-linked electrospun Siam weed gelatin scaffold by conducting tensile test3. The effect of concentration on solution properties, electrospinnibility and cell behavior were studied.

viii. The concentrations studied here include 25 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml.

ix. These Siam weed gelatin solutions were electrospun into nanofibrous scaffolds and their microstructure morphology was captured.

x. The solution properties investigated here include solution conductivity and solution degradation.

xi. The cell culture involved in this study was proliferation assay on chondrocytes cells.

1.5 Significant of the Study

The study of biomaterials and suitable microenvironment scaffolds is significant in tissue engineering application. In order to develop a successful 3D skin tissue engineering scaffold, the scaffold need to fulfill some specific requirements such as having adequate mechanical properties, biocompatibility characteristic and additional of therapeutic activities to enhance the healing process of skin damage due to burns and injury. This study involved developing a novel hydrogel hybrid using Siam Weed as its natural remedy to address the potential use of 3D skin grafts, Siam Weed and gelatin as an alternative to synthetic drugs commonly administrated to treat infected wounds.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Burn scars are a serious physical and even psychological problem for the affected people. The skin burn wound may be delayed and worst, becoming severely infected if not be treated properly in the manner of time [20]. Skin burn wound provide an easy access to the bacteria enabling located in the skin tissue to cause systemic processes and septic organ failure to worst [21]. Fortunately, thanks to current technology in tissue engineering, there are a few current studies on treatment of skin burn and injury. These treatment such as medical needling, non-cultured autologous skin suspension, small-fiber neuropathy induced by resiniferatoxin to promote mechanical and thermal hypoalgesia of the skin, and a novel CNE treatment against skin burn wound MRSA infections [1-3].

Basic scientific research has found that medical needling can improve the quality of burn scars with comparatively low risk and stress for the patient with regards to skin elasticity, moisture, erythema and transepidermal water loss. However, needling and small fiber neuropathy induced by resiniferatoxin treatment can increase risk of infection

and wound while the procedures are expansive and take much time to heal besides no details on its mechanical properties [1].

In the second chapter, the details of tissue engineering and hydrogel hybrid will be explained. Siam Weed compositions as its main materials to provide healing properties and mechanical properties by electrospinning will be further discussed to acknowledge the hydrogel hybrid primary behavior.

2.2 Tissue Engineering

Tissue engineering is an application of combining biological science and engineering in order to regenerate biological substitutes for replacing damaged organ or tissue [22]. The application involves three vital elements: that are cells, scaffold (3D polymeric matrix) and growth factors [23]. Scaffolds interacts with the cells and growth factor to regenerate specific tissue [24] therefore the scaffolds need to resembles the extracellular matrix that exists in our body and consists of 3D nanofibrous structure made of collagen and other biopolymers. Besides nanofibrous features, properties such as hydrophilicity, hydrophobicity, mechanical strength, biocompatibility, biodegradable and cell-matrix interaction greatly depend on the microstructure and the characteristic of polymer used for making tissue engineering scaffolds [23-25].

Above all, in order to produce a successful and excellent scaffold, the scaffold should mimic the morphological structure and chemical composition of the extracellular matrix (ECM) so that the cells can adhere to the scaffold surface, regenerate, proliferate and differentiate into new tissue [26]. Basic principles of tissue engineering are displayed in Figure 2.1 [27].

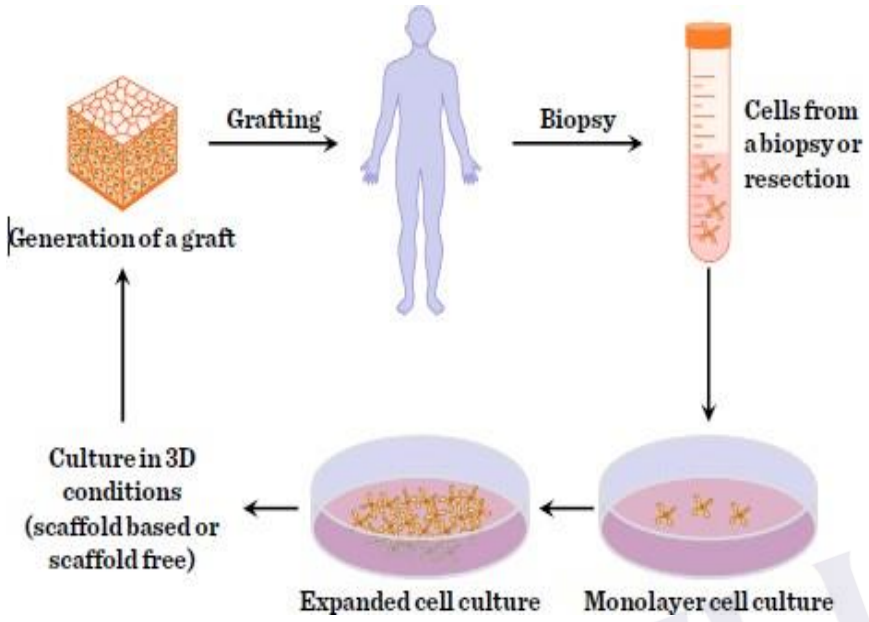

Figure 2.1: Basic principle of tissue engineering [27]

In tissue engineering scientific approach of replacing damaged skin from burns and injury, it is based on three important elements: cells, growth factors (signaling molecules) and scaffold (3D polymeric matrix). Specifically, scaffold is one of the most highlight where it interacts with cells and growth factor to regenerate tissues. Therefore, scaffolds need to fulfill certain requirements in order to develop successful scaffolds. The scaffolds need to imitate the extracellular matrix (ECM) that exists in body and provide microenvironment for the cells in order to govern the regeneration and proliferation of the cells [28]. The selection of materials and microstructure of the scaffolds affect the regeneration process [29]. Therefore, the materials used in the production of the scaffold need to be taken into close considerations.

One of the consideration is to choose a material with biocompatibility with living tissues. This defines the toleration of the materials where the materials do not produce a toxic or immunological response when exposed to the body or bodily fluids [30]. The scaffolds need to be accepted in host in which it planted to in order to gain positive responses and therefore the cells can function normally as it is in extra cellular matrix (ECM) as displayed in Figure 2.2. Biodegradability is also one of important function in

developing an excellent scaffold. Throughout time, tissue engineering field is looking for biodegradable materials that is harmless to the body and also can exit the body without interfering with body system. The implanted scaffold will biodegrade to allow body's own cell to replace it as they will produce their own extracellular matrix as the structural support.

Besides of biocompatibility and biodegradable materials, scaffolds also need to possess adequate mechanical properties in order to support cell attachment and growth. The mechanical properties of a scaffold are commonly determined by tensile properties. The fabrications of scaffolds also play an important role in developing an excellent scaffolds. For example, a 3D scaffold if made of nanofibers, should provide a biomimetic structure resembling the ECM [23]. The nano-scale features of a nanofibrous scaffold possess high surface to volume ratio, which enhances cell adhesion and cell proliferation, and even supply nutrient in a more efficient medium [31-32].

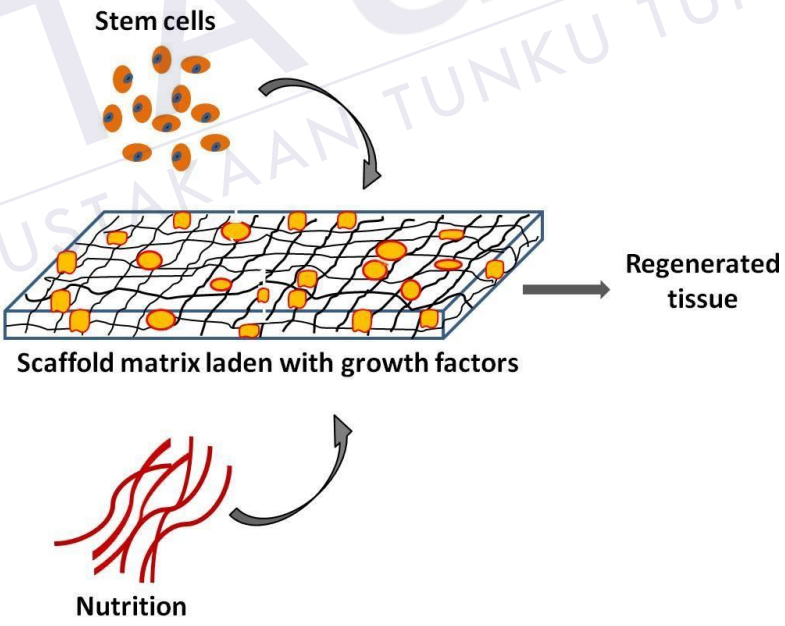

Figure 2.2: Scaffold for tissue engineering triad [33]

2.3 Biomaterials

In tissue engineering, the element of biomaterial is encompassed as it is considered as an alternative for synthetic drugs. In definition of a material that is natural or man-made, that comprises whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function [33]. Biomaterials should stimulate the natural extracellular matrix (ECM) and create a microenvironment that will suffice the tissue formation and function [34] Biomaterials can be classified into two groups: synthetic and natural biomaterials. Synthetic and natural biomaterials have their own advantage and disadvantages. Synthetic polymers are in a great control of structural architecture while mechanical properties and degradation rate are tunable. However, the use of synthetic polymer may results in body rejection due to reduced bioactivity [35] .

On the other hand, naturally derived biomaterials are far more risk-free as for their compatibility, bioactivity, degradability and even showing the same structural composition of the ECM. However, the limitation of the naturally derived biomaterial is lack of mechanical properties. In order to overcome the limitations, the idea of combining two groups of biomaterials in developing hybrid scaffold for example, wearable hydrogel in Figure 2.3 can be seen as an opportunity to develop an excellent scaffold with excellent characteristic [36].

One of the most commonly used biomaterials in tissue engineering is hydrogels. Hydrogels are 3D hydrophilic polymer network that can swell in aqueous solutions without dissolution, maintaining its structure [37-38].

Figure 2.3: Wearable Hydrogel [36]

2.3.1 Extracellular Matrix

The extracellular matrix (ECM) is a cellular component that exist within tissues and organs as displayed in Figure 2.4. ECM function is to supply essential physical scaffolding for the cells such as biochemical and biomechanical properties in microenvironment for the cells to regenerate, proliferate and migrate [38]. ECM consists of dynamic and complex array of glycoproteins, collagens, glycosaminoglycans and proteoglycans [39].

In the skin, ECM provides the dermis and the basement membrane in order to supply tensile strength and flexibility inherent to skin [39]. In addition, the structure of the ECM is highly dynamic as it constantly being remodeled, either enzymatically or non-enzymatically. ECM generates the biochemical and mechanical properties of each organ such as its tensile and compressive strength and elasticity. [40]

The ECM is composed of two main classes of macromolecules: proteoglycans (PGs) and fibrous proteins [41]. PGs are composed of glycosaminoglycan (GAG) chains covalently linked to a specific protein core. PGs have been classified according to their core proteins, localization and GAG composition. On the other hand, the main fibrous ECM proteins are collagens, elastins, fibronectins and laminins [42]. All of the molecules are secreted in the specific tissues. However, the composition and arrangement of the molecules differ with the type of tissues and changes according to the physiological form and state of the tissue.

ECM is very important in providing optimum microenvironment to the functional cells where it respond to change in molecular composition following to the tissue development. In tissue engineering, the functionality of ECM is imitated by biomaterials in providing microenvironment to the functional cells [43].

REFERENCES

[1] Busch K, Bender R, Walezko N, Aziz H, Altintas M, Aust M. Combination of medical needling and non-cultured autologous skin cell transplantation (ReNovaCell) for repigmentation of hypopigmented burn scars. *Burns*. 2016;42(7):1556-1566.

[2] Laverdet B, Girard D, Bayout A, Bordeau N, Demiot C, Desmoulière A. Effects of small-fiber neuropathy induced by resiniferatoxin on skin healing and axonal regrowth after burn. *Burns*. 2017;43(3):562-572.

[3] Song Z, Sun H, Yang Y et al. Enhanced efficacy and anti-biofilm activity of novel nanoemulsions against skin burn wound multi-drug resistant MRSA infections. *Nanomedicine:Nanotechnology,Biology.andMedicine*.2016;12(6): 1543-1555.

[4] Illeperuma W, Sun J, Suo Z, Vlassak J. Fiber-reinforced tough hydrogels. *Extreme Mech Lett*. 2014;1:90-96.

[5] Koh C, Kamarudin A, Khoo W, Mohamed N. Fiber-Hydrogel Composites for Skin Tissue Engineering. In: *2016 IEEE EMBS Conference On Biomedical Engineering And Sciences (IECBES)*. Kuala Lumpur, Malaysia: IEEE; 2016.

[6] Salgado A, Coutinho O, Reis R, Davies J. In vivo response to starch-based scaffolds designed for bone tissue engineering applications. *Journal of Biomedical Materials Research Part A*. 2007;80A(4):983-989.

[7] Yang S, Chen P, Chen Y, Lin F. Gelatin/chondroitin-6-sulfate copolymer scaffold for culturing human nucleus pulposus cellsinvitro with production of extracellular matrix. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2005;74B(1):488- 494.

[8] Sannino A, Madaghiele M, ConversanO F et al. Cellulose Derivative−Hyaluronic Acid-Based Microporous Hydrogels Crosslinked through Linked through Divinyl Sulfone (DVS) To Modulate Equilibrium Sorption Capacity and Network Stability. *Biomacromolecules*. 2004;5(1):92-96.

[9] Yannas I, Lee E, Orgill D, Skrabut E, Murphy G. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proceedings of the National Academy.of.Sciences*.1989;86(3):933-937.

[10] Pereira R, Mendes A, Bártolo P. Alginate/Aloe Vera Hydrogel Films for Biomedical Applications. *Procedia CIRP*. 2013;5:210-215.

[11] Pandey A, Mohan M, Singh P, Palni U, Tripathi N. Chemical composition, antibacterial and antioxidant activity of essential oil of Eupatorium adenophorum Spreng. from Eastern Uttar Pradesh, India. *Food.Bioscience*.2014;7:80-87.

[12] Zhao X, Liu D. Chemical and thermal characteristics of lignins isolated from Siam weed stem by acetic acid and formic acid delignification. *Industrial Crops and Products*. 2010;32(3):284-291.

[13] Abdulla M, Sidik K, Indran M, Suzainur K. Evaluation of in-vivo wound healing activity of Chromolaena odorata leaf extract on excision wounds model in rats. *Journal of Food Technology*. 2005;3(2):126-129.

[14] Pandith H, Zhang X, Thongpraditchote S, Wongkrajang Y, GritsanapanW, Baek S. Effect of Siam weed extract and its bioactive component scutellarein tetramethyl ether on anti-inflammatory activity through NF-κB pathway. *J Ethnopharmacol*. 2013;147(2):434-441.

[15] Pandith H, Zhang X, Ligget J, Min K, Gritsanapan W, Baek S. Hemostatic and Wound Healing Properties of Chromolaena odorata Leaf.Extract. *ISRN.Dermatol*.2013:168269.

[16] Zige D, Ohimain E, Nodu M. Antibacterial Activity of Ethanol, Crude and Water Extract of Chromolaena OdorataLeaves on S Typhi andEColi. *Greener Journal of Microbiology and Antimicrobials*.2013;1(2):016-019. doi:10.15580/gjma.2013.

[17] Huh D, Hamilton G, Ingber D. From 3D cell culture to organs-on- chips. *Trends Cell Biol*. 2011;21(12):745-754.

[18] Schmeichel K. Modeling tissue-specific signaling and organ function in three dimensions. *J Cell Sci*. 2003;116(12):2377-2388.

[19] Saji Joseph J, Tebogo Malindisa S, Ntwasa M. Two-Dimensional (2D) and Three-Dimensional (3D) Cell Culturing in Drug Discovery.

[20] Haisma E, de Breij A, Chan H et al. LL-37-Derived Peptides Eradicate Multidrug-Resistant Staphylococcus aureus from Thermally Wounded Human Skin Equivalents. *Antimicrob Agents Chemother*. 2014;58(8):4411-4419.

[21] Ottenio M, Tran D, Ní Annaidh A, Gilchrist M, Bruyère K. Strain rate and anisotropy effects on the tensile failure characteristics of human skin. *J Mech Behav Biomed Mater*. 2015;41:241-250.

[22] Zhang Q, Lv S, Lu J, Jiang S, Lin L. Characterization of polycaprolactone/collagen fibrous scaffolds by electrospinning and their bioactivity. *Int J Biol Macromol*. 2015;76:94-101.

[23] Gautam S, Dinda A, Mishra N. Fabrication and characterization of PCL/gelatin composite nanofibrous scaffold for tissue engineering applications by electrospinning method. *Materials Science and Engineering:C*. 2013;33(3):1228-1235.

[24] Ott H, Matthiesen T, Goh S et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med*. 2008;14(2):213-221.

[25] Powell H, Boyce S. Engineered Human Skin Fabricated Using Electrospun Collagen–PCL Blends: Morphogenesis and Mechanical Properties. *Tissue Engineering Part A*. 2009;15(8):2177-2187.

[26] Langer R, Vacanti J, Vacanti C, Atala A, Freed L, Vunjak- Novakovic G. Tissue Engineering: Biomedical Applications. *Tissue Eng*. 1995;1(2):151-161.

[27] Bartis D, Pongracz J. *Three Dimensional Tissue Cultures And Tissue Engineering*. University of Pécs; 2011.

[28] Hutmacher D, Loessner D, Rizzi S, Kaplan D, Mooney D, Clements J. Can tissue engineering concepts advance tumor biology research?. *Trends.Biotechnol*.2010;28(3):125-133.

[29] Liu X, Ma P. Polymeric Scaffolds for Bone Tissue Engineering. *Ann Biomed.Eng*.2004;32(3):477-486.

[30] Paleos G. What is Biocompatibility?. *Pittsburgh Plast Manufacturing*. 2012;510.

[31] Elsdale T. Collagen Substrata For Studies On Cell Behavior. *J Cell Biol.* 1972;54(3):626-637.

[32] Ma P, Zhang R. Synthetic nano-scale fibrous extracellular matrix. *J Biomed Mater Res*. 1999;46(1):60-72.

[33] Sharma S. Biomaterials in Tooth Tissue Engineering: A Review. *Journal Of Clinical And Diagnostic Research*. 2014.

[34] Tathe, A, Ghodke M, Nikalje A. A Brief Review: Biomaterials And Their Apllication. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2010;2(4).

[35] O'Brien, F. *Biomaterials & Scaffolds For Tissue Engineering*. 14th ed., 2011, pp. 88-95.

[36] Hoffman A. Hydrogels for biomedical applications. *Adv Drug Deliv Rev*. 2002;64:18-23

[37] Xing H, Lee H, Luo L, Kyriakides T. Extracellular matrix-derived biomaterials in engineering cell function. *Biotechnol Adv*. 2019:107421.

[38] Pasqui D, De Cagna M, Barbucci R. Polysaccharide-Based Hydrogels: The Key Role of Water in Affecting Mechanical Properties. *Polymers (Basel)*. 2012;4(3):1517-1534.

[39] Frantz C, Stewart K, Weaver V. The extracellular matrix at a glance. *J Cell Sci*. 2010;123(24):4195-4200.

[40] Venkatasubramanian, PN. *Imaging The Pancreatic ECM*. 2012, p. Chapter 2.

[41] Kim S, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *Journal of Endocrinology*. 2011;209(2):139- 151.

[42] Järveläinen H, Sainio A, Koulu M, Wight T, Penttinen R. Extracellular Matrix Molecules: Potential Targets in Pharmacotherapy. *Pharmacol Rev*. 2009;61(2):198-223.

[43] Monaghan, M. et al. *A Collagen-Based Scaffold Delivering Exogenous Microrna-29B To Modulate Extracellular Matrix Remodeling*. 22nd ed., 2014, pp. 786-796.

[44] Kim J, Park J, Kim J et al. Development of polyvinyl alcohol–sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *Int J Pharm*. 2008;359(1-2):79-86.

[45] Gombotz W. Protein release from alginate matrices. *Adv Drug Deliv Rev*. 1998;31(3):267-285.

[46] Clark, D. E and GreenH. C, "Alginic acid and process of making same.," *Pat. US2036922 A.*, 1936.

[47] Grant G, Morris E, Rees D, Smith P, Thom D. Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Lett*. 1973;32(1):195-198.

[48] Ahmed E. Hydrogel: Preparation, characterization, and applications: A review. *J Adv Res*. 2015;6(2):105-121.

[49] Apori, Long, Castro, Ã˜Rskov. Chemical composition and nutritive value of leaves and stems of tropical weed Chromolaena odorata. *Grass and Forage Science*. 2000;55(1):77-81.

[50] Alisi C, Ibegbulem C, Nwaogu, Cosmas U. Antimicrobial Action of Methanol Extract of Chromolaena Odorata-Linn is Logistic and Exerted by Inhibition of Dehydrogenase Enzymes. *Journal of Research in Biology*. 2011;3:209-219.

[51] Phan T, Wang L, See P, Grayer R, Chan S, Lee S. Phenolic Compounds of Chromolaena odorata Protect Cultured Skin Cellsfrom Oxidative Damage: Implication for Cutaneous Wound Healing. *Biol PharmBull*. 2001;24(12):1373-1379.

[53] Akinmoladun A, Ibukun E, Afor E, Obuotor E, Farombi E. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Academic Journals*. 2007;2(5):163-166.

[54] Sirinthipaporn A, Jiraungkoorskul W. Wound Healing Property Review of Siam Weed, *Chromolaena odorata*. *Pharmacogn Rev*. 2017;11(21):35–38.

[55] Patel J, Kumar G, Qureshi M, Jena P. Anthelmintic activity of Ethanolic extract of whole plant of Eupatorium odoratum. L. *International journal of phytomedicine*. 2010;2(2):127-132.

[56] Chakraborty A. Evaluation Of Analgesic Activity Studies Of Various Extracts Of Leaves Of Eupatorium Odoratum Linn. *International Journal Of Pharmacy And Technology*. 2010.

[57] Taiwo O, Olajide O, Soyannwo O, Makinde J. Anti-Inflammatory, Antipyretic And Antispasmodic Properties Of Chromolaena Odorata. *Pharm Biol*. 2000;38(5):367-370.

[58] Suksamrarn A, Chotipong A, Suavansri T et al. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of Chromolaena odorata. *Arch Pharm Res*. 2004;27(5):507-511.

[59] Phan T, Hughes M, Cherry G, Le T, Pham H. An aqueous extract of the leaves of Chromolaena odorata (formerly Eupatorium odoratum) (Eupolin) inhibits hydrated collagen lattice contraction by normal human dermal fibroblasts. *J Altern Complement Med*. 1996;2(3):335-343.

[60] Thang P, Patrick S, Teik L, Yung C. Anti-oxidant effects of the extracts from the leaves of Chromolaena odorata on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide andhypoxanthine– xanthine oxidase induced damage. *Burns*. 2001;27(4):319-327.

[61] Biswal P, Sardar K, Parija S, Mishra P. Wound healing effect of Eupatorium odoratum Linn. and Himax in rabbits. 1997;19:71-74.

[62] Wang C, Wang J, Zeng L, et al. Fabrication of Electrospun Polymer Nanofibers with Diverse Morphologies. *Molecules*. 2019;24(5):834.

[63] Chong E, Phan T, Lim I Et Al. Evaluation Of Electrospun PCL/Gelatin Nanofibrous Scaffold For Wound Healing And Layered Dermal Reconstitution☆. *Acta Biomater*. 2007;3(3):321-330.

[64] Luu Y, Kim K, Hsiao B, Chu B, Hadjiargyrou M. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA–PEG block copolymers. *Journal of Controlled Release*. 2003;89(2):341-353.

[65] Vasita R, Katti D. Nanofibers and their applications in tissue engineering. *Int J Nanomedicine*. 2006;1(1):15-30.

[66] Su Y, Nykanen M, Jahn KA, et al. Multi-dimensional correlative imaging of subcellular events: combining the strengths of light and electron microscopy. *Biophys Rev*. 2010;2(3):121–135. doi:10.1007/s12551-010-0035-2)

[67] Amiraliyan N, Nouri M, Kish M. Electrospinning of silk nanofibers. I. An investigation of nanofiber morphology and process optimization using response surface methodology. *Fibers and Polymers*. 2009;10(2):167-176

[68] Tomba E, Facco P, Roso M, Modesti M, Bezzo F, Barolo M. Artificial Vision System for the Automatic Measurement of InterfiberPore Characteristics and Fiber Diameter Distribution in Nanofiber Assemblies. *Ind Eng Chem Res*. 2010;49(6):2957-2968.

[69] Jacobs V, Anandjiwala R, Maaza M. The influence of electrospinning parameters on the structural morphology and diameter of electrospun nanofibers. *J Appl Polym Sci*. 2010;115(5):3130-3136.

[70] Thermofisher (2019) "Raman and FTIR Spectroscopy: Complementary Technologies"Retrieved on August 2019, from http://tools.thermofisher.com/content/sfs/brochures/Raman-FTIR.

[71] (Exline, D. (2020). Comparison of Raman and FTIR Spectroscopy: Advantages and Limitations | gateway analytical. [online] Gatewayanalytical.com.Availableat:https://www.gatewayanalytical.com/resources/publications/comparison-raman-and-ftir-spectroscopy-advantages-and-limitations [Accessed 14 Jan. 2020].

[72] Kishan, A. & Nezarati, R. & Radzicki, C. & Renfro, A. & Robinson, J. & Whitely, M. & Cosgriff-Hernandez, Elizabeth. (2015). Correction: In situ crosslinking of electrospun gelatin for improved fiber morphology retention and tunable degradation. J. Mater. Chem. B. 3. 10.1039/C5TB00937E..

[73] Chaitaworn, N. Master Thesis, Faculty of Engineering, Chulalongkorn University, 2009

[74] Ratanavaraporn J, Rangkupan R, Jeeratawatchai H, Kanokpanont S, Damrongsakkul S. Influences of physical and chemical crosslinking techniques on electrospun type A and B gelatin fiber mats. *Int J Biol Macromol*. 2010;47(4):431-438.

[75] Farina A, Fievet M, Plassart F, Menet M, Thuillier A. Residual glutaraldehyde levels in fiberoptic endoscopes: measurement and implications for patient toxicity. *Journal of Hospital Infection*. 1999;43(4):293-297.

[76] Van Luyn M, van Wachem P, Olde Damink L, Dijkstra P, Feijen J, Nieuwenhuis P. Relations betweenin vitro cytotoxicity and crosslinked dermal sheep collagens. *J Biomed Mater Res*. 1992;26(8):1091-1110.

[77] Huang-Lee L, Cheung D, Nimni M. Biochemical changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived crosslinks. *J Biomed Mater Res*. 1990;24(9):1185-1201.

[78] Simmons D, Kearney J. Evaluation of collagen cross-linking techniques for the stabilization of tissue matrices. *Biotechnol Appl Biochem*. 1993;17(1):23-9.

[79] Jayakrishnan A, Jameela S. Glutaraldehyde as a fixative in bioprostheses and drug delivery matrices. *Biomaterials*. 1996;17(5):471-484.

[80] Schmidt C, Baier J. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. *Biomaterials*. 2000;21(22):2215-2231.

[81] Skotak M, Noriega S, Larsen G, Subramanian A. Electrospun cross-linked gelatin fibers with controlled diameter: The effect of matrix stiffness on proliferative and biosynthetic activity of chondrocytes cultured in vitro. *Journal of Biomedical Materials Research Part A*. 2010;95A(3):828-836.

[82] Williams D. Tissue-biomaterial interactions. *J Mater Sci*. 1987;22(10):3421-3445.

[83] Konttinen Y, Zhao D, Beklen A et al. The Microenvironment around Total Hip Replacement Prostheses. *Clin Orthop Relat Res*. 2005;(430):28-38.

[84] Arshady, R.. Introduction to polymeric biomaterials. London: Citus Books, 2003.

[85] Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F, Nava- Saucedo J. Polymer biodegradation: Mechanisms and estimation techniques A review. *Chemosphere*. 2008;73(4):429-442.

[86] Kasper, C et al. *Tissue Engineering III: Cell - Surface Interactions For Tissue Culture.*. 2014, pp. 56-59.

[87] Lyu S, Untereker D. Degradability of polymers for implantable biomedical devices. *Int J Mol Sci*. 2009;10(9):4033–4065. Published 2009 Sep 11. doi:10.3390/ijms10094033)

[88] Kasper, C et al. *Tissue Engineering III: Cell - Surface Interactions For Tissue Culture.*. 2014, pp. 68-73.

[89] Jhon M, Andrade J. Water and hydrogels. *Journal of Biomedical Materials Research*. 1973;7(6):509-522. Mcm 80

[90] Liebschner M, Bucklen B, Wettergreen M. Mechanical Aspects of Tissue Engineering. *Semin Plast Surg*. 2005;19(3):217–228. doi:10.1055/s-2005-919717

[91] Roylance, D. *Mechanics Of Materials*. 2000.

[92] Lee KY, Mooney DJ. Alginate: properties and biomedical applications. *Prog Polym Sci*. 2012;37(1):106–126. doi:10.1016/j.progpolymsci.2011.06.003)

[93] Lim H, Hoag S. Plasticizer Effects on Physical–Mechanical Properties of Solvent Cast Soluplus® Films. *AAPS PharmSciTech*. 2013;14(3):903-910.

[94] "What Is Ultimate Tensile Strength?". *Science ABC*, 2020, https://www.scienceabc.com/pure-sciences/what-is-ultimate-tensile-strength.html. Accessed 8 July 2019.

[95] "Wounds And Injuries | Fracture | Bruises". *Medlineplus.Gov*, 2020, https://medlineplus.gov/woundsandinjuries.html. Accessed 12 Apr 2019.

[96] "Materials/Mechanical/Tensile". *Nde-Ed.Org*, 2020, https://www.nde-ed.org/EducationResources/CommunityCollege/Materials/Mechanical/Tensile.htm. Accessed 7 June 2019.

[97] Romar G, Kupper T, Divito S. Research Techniques Made Simple: Techniques to Assess Cell Proliferation. *Journal of Investigative Dermatology*. 2016;136(1):e1-e7.

[98] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1-2):55-63.

[99] Schafer K. The Cell Cycle: A Review. *Vet Pathol*. 1998;35(6):461-478.

[100] Romar, G., Kupper, T. and Divito, S. (2016). Research Techniques Made Simple: Techniques to Assess Cell Proliferation. *Journal of Investigative Dermatology*, 136(1), pp.e1-e7.)

[101] "Cell Proliferation: A Complete Guide". *Abcam.Com*, 2020, https://www.abcam.com/primary-antibodies/the-cell-proliferation-guide. Accessed 11 Nov 2019.

[102] Romar, G., Kupper, T. and Divito, S. Research Techniques Made Simple: Techniques to Assess Cell Proliferation. *Journal of Investigative Dermatology*, 2016:136(1), pp.e12-e16.)

[103] Smith. "Microplate Reader Assays For Cell Proliferation". *Biocompare.Com*, 2020, https://www.biocompare.com/Editorial-Articles/557885-Microplate-Reader-Assays-for-Cell-Proliferation. Accessed 16 May 2019.

[104] Romar, G., Kupper, T. and Divito, S. Research Techniques Made Simple: Techniques to Assess Cell Proliferation. *Journal of Investigative Dermatology*, 2016:136(1), pp.e10-e12.)

[105] Speight, J. Transformation of Inorganic Chemicals in the Environment. *Environmental Inorganic Chemistry for Engineers*, 2017, pp.333-382.

[106] Che Sulaiman IS, Basri M, Fard Masoumi HR, Chee WJ, Ashari SE, Ismail M. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. *Chem Cent J*. 2017;11(1):54.

[107] Song, J. H., Kim, H. E., & Kim, H. W. Production of electrospun gelatin nanofiber by water-based co-solvent approach. Journal of Materials Science: Materials in Medicine, 2008:19(1), 95–102.

[108] Butcher, A., Koh, C. and Oyen, M. Systematic mechanical evaluation of electrospun gelatin meshes. *Journal of the Mechanical Behavior of Biomedical Materials*, 2017:69, pp.412-419.

[109] Alharbi, Abdulaziz & Alarifi, Ibrahim & Khan, Waseem & Asmatulu, R.. Highly Hydrophilic Electrospun Polyacrylonitrile/ Polyvinypyrro-lidone Nanofibers Incorporated with Gentamicin as Filter Medium for Dam Water and Wastewater Treatment. 2016:5. 0-0

[110] Chung S, Khoo W. Strategy on the Production of Bead-Free Electrospun Gelatin Scaffolds. *Journal of Mechanical Engineering*. 2018;56:69-79.

[111] Dhand C, Balakrishnan Y, Ong ST, et al. Antimicrobial quaternary ammonium organosilane cross-linked nanofibrous collagen scaffolds for tissue engineering. *Int J Nanomedicine*. 2018;13:4473–4492.

[112] Yan, X., Zhou, P., Wang, F., Qiu, C., Wang, P., Zhang, Y., Zhao, L. and Xu, S. Degradability, biocompatibility, and osteogenesis of biocomposite scaffolds containing nano magnesium phosphate and wheat protein both in vitro and in vivo for bone regeneration. *International Journal of Nanomedicine*, Volume 11, 2016, pp.3435-3449.:

[113] Genesky G, Cohen C. Toughness and fracture energy of PDMS bimodal and trimodal networks with widely separated precursor molar masses. *Polymer (Guildf)*. 2010;51(18):4152-4159.

[114] Olde Damink L, Dijkstra P, Van Luyn M, Van Wachem P, Nieuwenhuis P, Feijen J. Glutaraldehyde as a crosslinking agent for collagen-based biomaterials. *Journal of Materials Science: Materials in Medicine*. 1995;6(8):460-472.

[115] Bhardwaj N, Kundu S. Electrospinning: A fascinating fiber fabrication technique. *Biotechnol Adv*. 2010;28(3):325-347.

[116] Geng X, Kwon O, Jang J. Electrospinning of chitosan dissolved in c oncentrated acetic acid solution. *Biomaterials*. 2005;26(27):5427-5432.

[117] Song J. Production of electrospun gelatin nanofiber by water-based co-solvent approach. *Journal of Materials Science: Materials in Medicine*. 2007;19(1):103-105.

[118] Tiwari S, Venkatraman S. Importance of viscosity parameters in electrospinning: Of monolithic and core–shell fibers. *Materials Science and Engineering: C*. 2012;32(5):1037-1042.

[119] Erencia M, Cano F, Tornero J, Macanás J, Carrillo F. Resolving the Electrospinnability Zones and Diameter Prediction for the Electrospinningof the Gelatin/Water/Acetic Acid System. *Langmuir*. 2014;30(24):7198-7205.

[120] Abdelrahman T, Newton H. Wound dressings: principles and practice. *Surgery (Oxford)*. 2011;29(10):491-495.

[121] Weller C, Sussman G. Wound Dressings Update. *Journal of Pharmacy Practice and Research*. 2006;36(4):318-324.

[122] Gallagher, A., Ní Anniadh, A., Bruyere, K., Otténio, M., Xie, H. and Gilchrist, M. 2012, http://www.ircobi.org/wordpress/downloads/irc12/pdf_files/59.pdf Accessed 18 Jan. 2019.